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Review

Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)

Ramey, R. R. II, H.-P. Liu, L. Carpenter

Submitted by Jeff Mitton to Gary Skiba

This study examines mtDNA sequence data and skull measurements of Preble's meadow jumping mouse, *Zapus hudsonius preblei*. The mtDNA sequences are compared to sequences from *Z. h. luteus*, *Z. h. pallidus*, *Z. h. campestris*, and the outgroups *Zapus princeps idahoensis*, *Z. p. princeps*, and *Z. p. utahensis*. Skull measurements are compared between *Z. h. preblei* and *Z. h. campestris*. Two important results emerge from these studies:

1) the haplotypes detected in *Z. h. preblei* are a subset of the haplotypes in *Z. h. campestris*--that is, the samples of *Z. h. preblei* did not reveal any unique haplotypes;

2) a discriminant function of skull measurements could only correctly classify 48% of the individuals to their correct subspecies—about the percentage (50%) that could be correctly assigned by random guessing.

Specific comments

Abstract line 3: change to analysis of skull measurements from 80...

Abstract line 6: change *campestris*, all to *campestris*; all

Page 3 change then previously to than previously

Page 3, 4th line up from the bottom. Hybridization with *Z. princeps* seems to come out of left field, and then is not mentioned again. Is this hybridization documented or speculated about in the literature? Why is it mentioned here? Will it be assessed in this report?

Page 5, first line—it will be unlikely that it will be differentiated for nuclear microsatellite DNA. This reviewer disagrees. The evolutionary rate of mtDNA is about 5 to 10 times as fast as nuclear genes in general, but nuclear microsatellites are a special case, for their mutation rates are far higher (in humans, frequently in the range .05 to .001) than rates in mtDNA. MtDNA should differentiate faster than most nuclear genes,

but microsatellites should differentiate faster than any other genetic marker.

Page 5, first paragraph: Once again, hybridization with *Z. princeps* is mentioned—this is confusing to the reader, who has not yet been given any explanation for considering hybridization.

Page 5, second paragraph—Is it sufficient to mention the crosshair classification described in Crandall et al (2000), or does this report need a brief description of the classification?

Page 5, last paragraph—The sequence of the primers should be included here. Alternatively, a reference to these primers should be given (preferably, both)

Page 6, second paragraph—same as the preceding comment

Page 7 The first two full sentences on this page are redundant

Page 8 Four *Z. h. preblei* were removed from this study; their mtDNA haplotypes were more similar to those of *Z. princeps princeps*, and the authors assumed that they were misidentified. (Or perhaps this is why hybridization with *Z. princeps* has been mentioned). This assessment should be given fuller treatment, for the deletion of *Z. h. preblei* haplotypes might cause some suspicion. One way around this is to include them in a tree, to show the critical reader that they are far away from *Z. h. preblei*, and in a clade with *Z. princeps*, supported by bootstrap values. Then the authors can assert that the samples were misidentified, or, alternatively, the *Z. princeps* haplotypes record hybridization with *Z. h. preblei*. The other deletions of data are less critical, for those subspecies are not being evaluated here.

Page 9, last full paragraph. This reviewer agrees. The most parsimonious assumption is that *Z. h. preblei* is simply an arm of the distribution of *Z. h. campestris*, and therefore contains a subset of the variation in *Z. h. campestris*.

Page 13, first line. “The lack of genetic, morphological or published ecological evidence for genetic distinctiveness (including adaptive divergence)...” It sounds like there have not been any comparative studies of the life history variation or habitats of *Z. h. campestris* and *Z. h. preblei*. Is that the case? Either way, the conclusion is not changed, but it provides more information to the critical reader to report that “no studies have been performed” versus “comparative studies have not revealed differences.”

Page 13 “significant gap in the range of a taxon...”. This could not be evaluated by this reviewer, for the copy of the report contained only figure 2, no figure 1, no figure 3.

Specific Questions

#1. Yes, appropriate markers and methods were used.

#2 I have suggested some revisions, including a more explicit treatment of the 4 *Z. h. preblei* removed from this study. If it is generally agreed that those 4 samples contained mtDNA from *Z. princeps*, then yes, I believe that the conclusions in this report are logical and defensible.

#3 If the removal of the 4 samples mentioned in #3 is prudent and appropriate, then I do not think the data have another parsimonious explanation.

#4 Additional observations: either misidentification of species and subspecies is not uncommon, or hybridization among taxa is not uncommon. These hypotheses could be tested with nuclear markers.

#5 I did not note any interpretation of the mechanism of reduced gene flow. In fact, Ramey et al reported that all of the haplotypes in *Z. h. preblei* were found in *Z. h. campestris*. Note that the figure of geographic distributions was not included in the review copy, so this reviewer could not assess or appreciate the proximity of the geographic distributions

#6—Do you agree with the concepts of Crandall et al (2000) for defining evolutionary significant units?

In general, I do agree. The reliance on both genetic and ecological exchangeability has a lot of biological intuition behind it. Unfortunately, the number of studies considering substantial genetic and ecological data are really quite small. For example, in the present study of *Zapus*, that all populations are adjacent to streams, and that “A review of the literature reveals that no quantitative evidence exists to reject the hypotheses of historic or recent ecological exchangeability...”. That is, no one has reported adaptive or ecological differences, but it is not clear that anyone has looked. Thus, we can fill in minus signs on the upper and lower right of the crosshair classification (Table 1 and Figure 1 in Crandall) but I think these are the necessary presumptions that you make in the absence of rigorously collected, comparative data.

#7 No.